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Note

Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry for species identification of Nonfermenting Gram-Negative Bacilli

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ABSTRACT

Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) to identify 396 Nonfermenting Gram-Negative Bacilli clinical isolates was evaluated in comparison with conventional phenotypic tests and/or molecular methods.
MALDI-TOF MS identified to species level 256 isolates and to genus or complex level 112 isolates. It identified 29 genera including uncommon species.

Most of the studies published about Nonfermenting Gram-Negative Bacilli (NFGNB) identification by MS refer to species mainly isolated from respiratory secretions of patients suffering from cystic fibrosis (CF) (Vanlaere et al., 2008; Degand et al., 2008; Marko et al., 2012; Fernández-Olmos et al., 2012, Lambiase et al., 2013). This study assesses the ability of MALDI-TOF MS to identify other species of NFGNB, including uncommon species.

We evaluated MALDI-TOF MS performance identification of 396 NFGNB clinical isolates recovered between 2009 and 2013 from clinical samples at the Hospital de Clínicas José de San Martín, Universidad de Buenos Aires, Argentina. The analysis was performed using Bruker Daltonics MicroFlex LT instrument, Biotyper software 3.1 (Bruker Daltonics, Bremen, Germany). This study included 29 different genera of NFGNB. All isolates from clinically significant samples were previously identified using standard biochemical tests following the identification scheme proposed by Wauters and Vaneechoutte (2011). In addition, 16S rRNA gene sequencing (Weisburg et al., 1991) and *recA* gene (1040 bp) sequencing (Mahenthiralingam et al., 2000) were used on 91 isolates and 28 *Burkholderia cepacia* complex (BCC) isolates, respectively.

Additionally, 20 *Acinetobacter baumannii* and 34 non-*baumannii* *Acinetobacter* spp. isolates were characterized by sequence analysis of *rpoB* using the primers previously described (Gundi et al., 2009).

All PCR reactions were carried out with *Taq* DNA polymerase based on manufacturer's specifications (Promega). Sequencing of PCR products was performed on both DNA strands using ABIPrism 3100 BioAnalyzer equipment at Macrogen Inc., South Korea. The sequences were analyzed using the BLAST v2.0 software (http://www.ncbi.nlm.nih.gov/BLAST/).

A colony growth was smeared on MALDI target and overlaid with 1.5 μl of formic-acid (70%), air-dried and overlaid with 1 μl of matrix (α-cyano-4-hydroxycinnamic acid). Mass spectra were acquired using the MALDI-TOF MS spectrometer in a linear positive mode (Microflex, Bruker Daltonics). A bacterial test standard was used for instrument calibration. Each specimen was run in duplicate and mass spectra were analyzed in an *m/z* range of 2000 to 20,000.

For result interpretation, the score cutoffs recommended by the manufacturer were used to determine species-level identification (≥2.000), genus/family/group-level identification, 1.7 to 1.999, and <1.700, unreliable identification. In addition, a 10% difference between the first two best matches in the database was required to give species identification. If this condition were not met, identification was considered correct at the genus or group level if the first two matches belonged to the same genus or group of bacteria. In this study,

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misidentification was defined as a discrepancy between the identification result given by MALDI-TOF MS and the result obtained by biochemical conventional test and/or by 16S rRNA or *recA* or *rpoB* sequencing (Saffert et al., 2011). MALDI-TOF identification was considered correct when the result obtained from MS database agreed with the phenotypic or molecular identification result.

In the case of *Pseudomonas fluorescens* complex (*P. fluorescens*, *Pseudomonas lundensis*, *Pseudomonas libanensis*, *Pseudomonas koorensis*)

Table 1
Agreement of MALDI-TOF MS with standard biochemical or molecular identifications.

Conventional or molecular identification method	No. of isolates	No. of isolates in agreement with			
		Species level	Genus/complex level	Misidentified	Unreliable identification
Achromobacter spp.	48		48[a]		
Acinetobacter spp.					
A. baumannii	20	19		1[b]	
A. johnsonii	6	5	1		
A. lwoffii	3	3			
A. pittii	11	11			
A. ursingii	3	2		1[c]	
A. junii	3				
A. radioresistans	1	3			
A. guilloiae	1	1			
A. haemolyticus	2	1			
A. oleivorans	1	2		1[d]	
A. soli	1			1[e]	
Alcaligenes faecalis	6	6			
Bordetella bronchiseptica	3	3			
Bordetella holmesii	2	2			
Bordetella parapertussis	1	1			
Bordetella hinzii	3	3			
Bordetella trematum	3	3			
Brevundimonas diminuta	8	8			
Brevundimonas vesicularis	1	1			
Burkholderia cepacia complex					
B. cenocepacia	7	7			
B. cepacia	6	2		4[f]	
B. lata	1		1[g]		
B. contaminans	12	4	7[h]	1	
B. multivorans	1	1			
B. vietnamiensis	1	1			
Burkolderia gladioli	6	5			1
Chryseobacterium gleum	4	4			
Chryseobacterium indologenes	3	1	1[i]	1	
Comamonas kerstersii	10	10			
Comamonas testosteroni	1	1			
Cupriavidus pauculus	1	1			
Cupriavidus respiraculi	1	1			
Delftia acidovorans	6	6			
Elizabethkingia meningoseptica	13	9		4[j]	
Empedobacter brevis	1	1			
Inquilinus limosus	4	2			2
Kerstersia gyorium	2	2			
Myroides odoratimimus	6	6			
Ochrobactrum antrophi	7	2	5[k]		
Oligella urethralis	10	10			
Pseudomonas oryzihabitans	3	3			
Pandoraea apista	1	1			
Pandoraea pulmonicola	1	1			
Pandoraea sputorum	2	2			
Pannonibacter phragmitetus	6	6			
Pseudomonas aeruginosa	24	24			
Pseudomonas mendocina	4	4			
Pseudomonas stutzeri	13	13			
Pseudomonas putida group	34		34[l]		
Pseudomonas fluorescens group	15		15[m]		
Ralstonia mannitolilytica	2			2[n]	
Ralstonia pickettii	2	2			
Rhizobium radiobacter	5	5			
Stenothrophomonas maltophilia	25	25			
Shewanella algae	9	1		8[o]	
Shewanella putrefaciens	4	4			
Sphingobacterium multivorum	9	9			
Sphingobacterium spiritivorum	1	1			
Sphingomonas paucimobilis	2	1			1
Wautersiella falsenii	2	2			
Weeksella virosa	1	1			
Wohlfahrtiimonas chitiniclastica	1	1			
Total (% agreement to level)	396	256 (64.65%)	112 (28.28%)	24 (6.06%)	4 (1.01%)